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RESEARCH NOTE

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Evaluation for Efficacies of Commercial Sanitizers and Disinfectants against *Bacillus cereus* Strains

Il Jin Kim, Ji-Hyoung Ha, Yong-Su Kim[1], Hyung-Il Kim[2], Hyun-Chul Choi[2], Dea-Hoon Jeon[2], Young-Ja Lee[2], Ae Jung Kim[3], Dong-Ho Bae[4], Keun-Sung Kim, Chan Lee, and Sang-Do Ha*

Department of Food Science and Technology, Chung-Ang University, Anseong, Gyeonggi 456-756, Korea
[1]*Korea Health Industry Development Institute, Seoul 156-800, Korea*
[2]*Korea Food & Drug Administration, Seoul 122-704, Korea*
[3]*Department of Food and Nutrition, Hyejeon College, Hongseong, Chungnam 350-702, Korea*
[4]*Department of Bioscience and Biotechnology, Konkuk University, Seoul 143-701, Korea*

Abstract Bactericidal efficacies of various sanitizers and disinfectants against 10 *Bacillus cereus* strains isolated from Korean foods and 8 standard *B. cereus* strains were investigated. The sanitizing capabilities of ethanol, iodine, chloride, quaternary ammonium, hydrogen peroxide, and peroxide acetic acid were investigated using the EN 1276 method based on quantitative suspension testing. The resistance against sanitizers and disinfectants was higher for wild-type than standard strains, and the bactericidal activities decreased in dirty conditions. Ethanol, chlorine, and iodine at the maximum level allowed under Korean food sanitation laws showed a great effectiveness against *B. cereus.* Hydrogen peroxide at 1,100 ppm showed the lowest bactericidal activity against *B. cereus*. These results indicate that the legally allowed maximum concentrations of sanitizers and disinfectants in Korea do not reduce all *B. cereus* strains by at least 5 $\log_{10}$ CFU/mL.

Keywords: efficacy, sanitizer, disinfectant, food borne pathogen, *Bacillus cereus*

Introduction

Controlling microorganisms that cause food-borne illnesses is important to food safety, and increasing attention is being paid to physical, chemical, and biological methods for controlling food-borne pathogens, especially in food processing plants. Physical methods include high-voltage pulsed electric fields (1,2), oscillating magnetic fields, high hydrostatic pressures (3), sonication, and microwave treatment (4). Chemical methods include the use of disinfectants and sanitizers such as alcoholic compounds (5), quaternary ammonium compounds, iodine, acid/alkali solutions (6), and surfactants. Most food processing plants tend to employ chemical methods due to their greater convenience and lower cost. The use of chemical disinfectants is expected to increase in order to reduce the incidence of food poisoning associated with contact surfaces of cafeterias and other food-processing facilities. In July 2007 there were 176 chemical sanitizers and disinfectants legally registered by the Korea Food & Drug Administration (KFDA) using standard method. Products that reduce *Escherichia coli* ATCC 10536 (also known as ATCC 11229) and *Staphylococcus aureus* ATCC 6538 by at least 99.999% (5 $\log_{10}$ CFU/mL) are approved for use. The evaluation methods do not consider food-borne pathogenic bacteria other than *S. aureus* and *E. coli*. This research chose *Bacillus cereus*, which is widely found in soil (7-10) and possesses strong resistance against chemical sanitizers. The efficacies of approved *B. cereus* - sanitizers and disinfectants sold in the Korean market were evaluated in this research.

*Corresponding author: Tel: +82-31-6704831; Fax: +82-31-6754853
E-mail: sangdoha@cau.ac.kr
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Materials and Methods

Bacterial samples In this experiment, 10 strains of wild-type *B. cereus* isolated from brown rice, barley, glutinous rice, humans, adlay flour, and white rice were obtained from the Food Safety Research Laboratory at Kangwon National University. The wild-type strains of *B. cereus* were identified by API (API 50 CHB/E medium; bioMerieux, Craponne, France) before being used in the experiments. The following 8 standard strains were also used: ATCC 14893, 53522, 21772, and 11778; KCTC 1092, 1094, and 1013; and KFRI 181. The bacteria were preserved and cultivated by spreading the sample strains on tryptic soy agar (TSA, Difco Laboratories, Detroit, MI, USA) and incubating them for 18-24 hr. Active cultivation was applied to 2 other sample sets.

Sanitizers and disinfectants Table 1 lists the 10 sanitizers and disinfectants that were purchased from a Korean market to evaluate their efficacy against the bacteria. The selected treatment concentrations were maximally allowed concentrations against food processing machine and utensils by KFDA.

Evaluation of efficacies of sanitizers and disinfectants The efficacy of the sanitizers and disinfectants were tested using the European EN 1276 method based on quantitative suspension testing (11,12). KFDA also uses EN method as the official method. Eight mL of disinfecting product was

Interfering substance (1 mL) + test strain (1 mL)

↓

20±1°C, 180±10 sec

↓

Disinfecting product (8 mL)

↓

20±1°C, 300±10 sec

↓

Disinfecting product (1 mL) + neutralizing agent (8 mL) + sterile water (1 mL)

↓

20±1°C, 300±10 sec

↓

Neutralized mixture (1 mL) + TSA plate (15 mL)

↓

36±1°C, 24 hr

↓

Counting

Fig. 1. Dilution and neutralization protocol.

added to a mixture containing 1 mL of the test strain and 1 mL of interfering substance. After the mixture reacted at 20±1°C (mean±SD) for 5 min and was agitated, 1 mL of the mixture was taken and added to a mixture containing 8 mL of neutralizing agent and 1 mL of sterile water. This mixture was then maintained for 5 min at 20±1°C to ensure complete neutralization, after which 1 mL of the mixture was immediately applied to a sterilized petri dish, with TSA used to count the live bacteria (Fig. 1).

Interfering substances Sterile interfering substance samples were prepared by resolving 0.3 g of bovine serum albumen (BSA; Sigma-Aldrich, St. Louis, MO, USA) into 100 mL of water and then filtering it through a membrane filtration system prior to use (0.45-μm pore diameter, AG 3770770; Sartorius, Gottingen, Germany). Interfering-substance samples in dirty conditions were prepared by using 3 g of resolved BSA in 100 mL of water.

Neutralizing agent The neutralizing agent was created by combining 3 g of lecithin (Fluka, Switzerland), 30 g of polysorbate 80 (Fluka), 5 g of sodium thiosulfate (Sigma-Aldrich), 1 g of L-histidine (Sigma-Aldrich), and 30 g of saponin (Fluka, Germany) in a 1,000-mL flask. The mixture was then diluted with a diluting agent to increase its mass, resolved, and sterilized prior to use.

Diluting agent The solution for dilution was prepared by resolving 1 g of tryptone and 8.5 g of NaCl into 1,000 mL of distilled water. This solution was then sterilized before use.

Hard water Solution A was prepared by resolving and sterilizing 19.84 g of $MgCl_2$ and 46.24 g of $CaCl_2$ to create a total volume of 1,000 mL. Solution B was prepared by resolving 35.02 g of $NaHCO_3$ into water and filtering through a membrane filtration system. The hard water used in sanitizers and disinfectants was created by adding 6.0 mL of solution A to at least 600 mL of sterile water in a 1,000-mL flask, to which 8 mL of solution B was added, with sterile water then added to make up a total volume to 1,000 mL.

Bacterial sample suspension The bacterial sample suspension was created by adding 10 mL of sterilized diluting agent and 5 g of sterilized glass beads to a 100-mL triangular flask. The activated trial sample of bacteria was inoculated with white gold. A colorimeter (Vitek; HACH, Loveland, CO, USA) revealed that the initial bacterial population was 1.5-2.5×10^8 CFU/mL. The sample was then maintained at a constant temperature (20±1°C) for 2 hr before use. The bacterial population in the resulting bacterial sample suspension was measured with TSA to produce the desired population size.

Results and Discussion

The results of evaluating 10 sanitizers and disinfectants using the quantitative suspension method are listed in Table

Table 1. Tested commercial sanitizers and disinfectants

Sanitizer/disinfectant	Active ingredient	Concentrations of active ingredient (%)	Treatment concentration
P-1	Ethanol	95.0	95.0%
P-2	Ethanol	75.0	75.0%
P-3	Hypochlorous acid	4.0	200 ppm[1)]
P-4	Sodium dichloroisocyanurate	94.0	100 ppm
P-5	Iodine	2.0	15 ppm
P-6	Iodine	1.5	25 ppm[1)]
P-7	Quaternary ammonium	5.0	100 ppm[1)]
P-8	Acetic acid/sulfuric acid	14.0/9.8	280 ppm/196 ppm
P-9	Peroxyacetic acid	5.8	145 ppm
P-10	Hydrogen peroxide	35.0	1,100 ppm[1)]

[1)]The selected treatment concentrations are maximum allowed concentration against food processing machine and utensils by KFDA.

Table 2. Efficacies of 10 sanitizers and disinfectants against *B. cereus* strains

Condition		Test organism	Reduction of *B. cereus*[1)] (log_{10} CFU/mL)									
			P-1	P-2	P-3	P-4	P-5	P-6	P-7	P-8	P-9	P-10
Clean	Standard *B. cereus*	ATCC 14893	>5	>5	>5	>5	4.72	>5	>5	>5	>5	1.71
		ATCC 53522	>5	>5	>5	>5	1.84	>5	>5	>5	>5	3.65
		ATCC 21772	>5	>5	>5	>5	2.91	>5	>5	>5	>5	1.91
		ATCC 11778	>5	>5	>5	>5	>5	>5	>5	>5	>5	>5
		KCTC 1092	>5	>5	>5	>5	3.24	>5	>5	>5	>5	2.83
		KCTC 1094	>5	>5	>5	4.64	4.00	>5	>5	>5	>5	2.93
		KCTC 1013	>5	>5	>5	4.03	3.58	4.97	>5	>5	>5	3.00
		KFRI 181	>5	>5	>5	>5	4.27	>5	>5	>5	>5	3.04
	Wild-type *B. cereus*	Brown rice 1	>5	>5	4.88	4.88	3.47	>5	4.85	4.88	4.81	2.44
		Brown rice 2	3.83	3.88	3.86	3.95	3.48	4.12	3.86	4.02	3.83	2.03
		Barley 1	>5	>5	>5	>5	3.59	>5	>5	>5	>5	1.82
		Barley 2	>5	>5	>5	>5	3.70	>5	>5	>5	>5	1.22
		Glutinous rice 1	>5	>5	>5	>5	3.42	4.37	3.78	4.01	4.45	1.39
		Glutinous rice 2	>5	>5	>5	>5	3.76	>5	>5	>5	>5	1.64
		Human 1	>5	>5	>5	>5	4.13	>5	>5	>5	>5	2.20
		Human 2	>5	>5	>5	>5	2.66	4.31	3.33	>5	>5	1.46
		Adlay flour	>5	>5	>5	>5	3.13	>5	>5	>5	>5	1.83
		White rice	4.52	4.51	4.39	4.36	2.22	4.43	4.27	4.27	4.32	2.35
Dirty	Standard *B. cereus*	ATCC 14893	>5	>5	3.81	4.31	3.66	>5	>5	>5	>5	1.57
		ATCC 53522	>5	>5	2.62	>5	1.44	4.61	>5	>5	>5	2.26
		ATCC 21772	>5	4.42	3.22	1.65	0.84	3.22	2.00	>5	>5	0.34
		ATCC 11778	>5	>5	>5	>5	>5	>5	>5	>5	>5	4.81
		KCTC 1092	>5	>5	4.49	2.49	1.19	4.90	4.71	>5	>5	2.06
		KCTC 1094	>5	>5	3.69	4.40	1.88	>5	>5	>5	>5	2.93
		KCTC 1013	>5	>5	>5	3.16	1.48	4.24	>5	>5	>5	3.00
		KFRI 181	>5	>5	3.91	4.93	1.26	4.96	>5	>5	>5	3.04
	Wild-type *B. cereus*	Brown rice 1	4.89	>5	4.88	1.70	2.20	2.48	4.80	4.70	4.82	1.15
		Brown rice 2	4.02	3.94	3.83	3.82	2.96	3.08	3.83	3.82	3.83	1.49
		Barley 1	>5	>5	3.76	2.84	2.70	>5	>5	>5	>5	0.39
		Barley 2	>5	>5	3.83	3.49	2.83	3.57	>5	>5	>5	1.51
		Glutinous rice 1	3.15	3.30	>5	3.76	2.60	4.39	1.60	4.07	4.40	0.53
		Glutinous rice 2	>5	>5	4.65	3.60	3.14	2.85	>5	>5	>5	1.53
		Human 1	>5	>5	1.65	2.31	1.05	>5	>5	>5	>5	1.50
		Human 2	>5	>5	>5	>5	2.66	4.31	1.57	>5	>5	0.98
		Adlay flour	>5	>5	3.09	3.09	2.93	2.61	4.50	>5	>5	0.65
		White rice	4.38	4.28	4.28	4.25	0.83	3.46	4.38	4.31	4.38	1.45

[1)]Initial concentration of *B. cereus*: 2×10^8 (8.414 log) CFU/mL.

2. Sanitizers and disinfectants containing 75 and 95% ethanol reduced all standard *B. cereus* strains except for ATCC 21772 by more than 5 log_{10} CFU/mL in dirty conditions. Application of the sanitizers and disinfectants reduced the 2 wild-type *B. cereus* strains originating from rice by 3.83-4.52 log_{10} CFU/mL, whereas all the other wild-type *B. cereus* strains were reduced by at least 5 log_{10} CFU/mL in clean conditions. In dirty conditions, 3 wild-type strains were reduced by 3.15-4.38 log_{10} CFU/mL, whereas all the other wild-type strains were reduced by more than 5 log_{10} CFU/mL. The results of using the 2 types of sanitizers and disinfectants containing chlorinated organic products and chlorinated inorganic products at their legally allowed maximum concentrations according to 'no-rinse' standards are listed in Table 2. Although using P-3 in clean conditions reduced all but 2 strains from brown rice and white rice by more than 5 log_{10} CFU/mL, such a reduction was only induced by P-3 in dirty conditions against ATCC 11778, KCTC 1013, and 2 wild-type strains from glutinous rice and humans. Peng *et al.* (13) reported that when *B. cereus* could not form spores and was in a vegetative state, 25 ppm sodium hypochlorite reduced more than 5 log_{10} CFU/mL within 15 sec. The inorganic chlorinated sanitizer, P-4, reduced KCTC 1094, KCTC 1013, 2 strains from brown rice, and 1 strain from white rice by more than 5 log_{10} CFU/mL in clean conditions. However, the P-4 tested in dirty conditions reduced all strains by only 1.65-4.31 log_{10} CFU/mL. Two iodine products (P-5 and P-6) were also

tested. P-5 (containing 15 ppm iodine) reduced all *B. cereus* strains by less than 5 $\log_{10}$ CFU/mL in both clean and dirty conditions with an exception of ATCC 11778, and P-6 (containing 25 ppm of iodine, which is the maximum lawful concentration) also reduced all *B. cereus* strains by less than 5 $\log_{10}$ CFU/mL in dirty conditions. The quaternary ammonium compound, P-7, reduced 5 wild-type *B. cereus* strains isolated from brown rice, glutinous rice, humans, and 1 from white rice by less than 5 $\log_{10}$ CFU/mL in clean conditions. In dirty conditions, P-7 reduced ATCC 21772, KCTC 1092, and the wild-type stains from brown rice, glutinous rice, humans, adlay flour, and white rice by less than 5 $\log_{10}$ CFU/mL. Although the resistance of quaternary ammonium against bacteria has been widely reported (14-16), the results are generally not related to the practical commercialized sanitizers used in food facilities. Peng *et al.* (13) reported that when *B. cereus* could not form spores and was in a vegetative state, a quaternary ammonium compound at 100 ppm reduced it by more than 5 $\log_{10}$ CFU/mL within 15 sec. The acid-type sanitizer, P-8, reduced all standard *B. cereus* strains by more than 5 $\log_{10}$ CFU/mL in both clean and dirty conditions, but this level of reduction was only evident against 6 wild-type strains in clean and dirty conditions, with other 4 wild-type strains showing reductions of 3.82-4.88 $\log_{10}$ CFU/mL. Although the peroxyacetic acid sanitizer, P-9, reduced 4 wild-type *B. cereus* strains by less than 5 $\log_{10}$ CFU/mL, its efficacy was generally higher than those of the other compounds. The hydrogen peroxide sanitizer, P-10, showed the lowest efficacy among all 10 sanitizers and disinfectants, reducing most of the strains of *B. cereus* by only 1-3 $\log_{10}$ CFU/mL in clean and dirty conditions. *B. cereus* exhibits a high resistance to disinfectants, with sanitizers reducing it by only 4-5 $\log_{10}$ CFU/mL. This contrasts with the 10 tested sanitizers and disinfectants being approved by the KFDA as reducing *E. coli* and *S. aureus* strains by more than 5 $\log_{10}$ CFU/mL.

The results of this research indicate that the approved products do not reduce all standard and wild-type *B. cereus* strains by at least 5 $\log_{10}$ CFU/mL. It therefore appears that the approval system for sanitizers and disinfectants underlying Korean food sanitation laws needs to be revised.

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